

CLAIMS

1. A method of producing a *thy A*⁻ strain of *Vibrio cholerae* comprising the
5 step of site-directed mutagenesis in the *V. cholerae* chromosome for the deletion and/or
insertion of gene nucleotides at the locus of the *thy A* gene having essentially the
nucleotide sequence SEQ ID NO: 1 of FIG. 1.
2. A *Vibrio cholerae thy A*⁻ strain which is a Δ *thy A* strain lacking the
functionality of the *thy A* gene.
- 10 3. A Δ *thy A* strain of *Vibrio cholerae* according to claim 2 comprising one
or several episomal autonomously replicating DNA elements having a functional *thy A*
gene that enables the strain to grow in the absence of thymine in the growth medium.
4. A Δ *thy A* strain of *Vibrio cholerae* according to claim 3, wherein the
episomal autonomously replicating DNA element is a plasmid.
- 15 5. A Δ *thy A* strain of *Vibrio cholerae* according to claim 3 or 4 comprising
a foreign *thy A* gene.
6. A Δ *thy A* strain of *Vibrio cholerae* according to claim 5, wherein the
foreign *thy A* gene is an *E. coli* gene.
7. A Δ *thy A* strain of *Vibrio cholerae* according to any one of claims 3 to
20 6, wherein the one or several episomal autonomously replicating DNA elements also
comprise a structural gene encoding a homologous or heterologous protein.
8. A Δ *thy A* strain of *Vibrio cholerae* according to claim 7, wherein the
encoded protein is selected from heat labile enterotoxin B-subunit of *Escherichia coli*
(LTB) and *Schistosoma japonicum* glutathione S-transferase 26 kD protein (GST 26
25 kD).
9. A nucleotide sequence of a *thy A* gene of *Vibrio cholerae* having
essentially the nucleotide sequence SEQ ID NO: 1 of FIG. 1.
10. A nucleotide sequence of a 5'-flanking region of a structural *thy A*
gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 2 of
30 FIG. 2.
11. A nucleotide sequence of a 3'-flanking region of a structural *thy A*
gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 3 of
FIG. 3.

12. A protein encoded by a nucleotide sequence of a *thy A* gene of *Vibrio cholerae* according to claim 9.

13. A protein according to claim 12, wherein the protein has the amino-acid sequence SEQ ID NO: 4 of FIG. 4.

5 14. A protein encoded by a nucleotide sequence of a 5'-flanking region of a structural *thy A* gene of *Vibrio cholerae* according to claim 10.

15. A protein according to claim 14, wherein the protein has the amino-acid sequence SEQ ID NO: 5 of FIG. 5.

10 16. A vaccine comprising as an immunising component a *Vibrio cholerae* Δ *thy A* strain according to any one of the claims 2 - 8 or a *thy A*⁻ strain of *Vibrio cholerae* produced by the method of claim 1.